FLIPPING THE SWITCH ON PD-1 IN MYELOMA

BY

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ABSTRACT

Marrow infiltrating lymphocytes (MILs) represent a novel source of T-cells for adoptive immunotherapy. In multiple myeloma (MM) MILs are derived from the bone marrow (BM) which is also represents the tumor microenvironment. Unlike peripheral blood lymphocytes (PBLs), MILs have an enriched central memory T-cell population, demonstrate intrinsic tumor-specificity and home to the bone marrow upon re-infusion. While MILs have demonstrated measurable clinical responses, many patients eventually relapse. This may be due to MILs succumbing to tumor-induced anergy. To overcome this, PD-1 blockade which has been used extensively clinically with impressive clinical results with many solid tumors, represents an approach to prevent tolerance and enhance T cell activity. As a single agent, PD-1 blockade has not shown any clinical benefit in patients with MM. PD-1 blockade primarily engages PD-1 on T-cells to prevent tumor-induced anergy. However, MM tumor cells also express PD-1 and this expression may contribute to the lack of responses observed with PD-1 blockade in MM since the true role of PD-1 on MM cells is unknown and may represent a pathway that restricts tumor outgrowth. If PD-1 engagement could be targeted directly to T-cells, it may then increase its therapeutic benefit in MM. To do this, a lentiviral PD-1/4-1BB (PD1BB) switch receptor was made. By over-expressing the PD1BB switch receptor on the surface of T-cells, it will effectively out-compete naturally expressed PD-1 for binding to its ligands, PD-L1 and PD-L2. Not only will this prevent an inhibitory signaling

cascade from naturally expressed PD-1 leading to anergy, but upon binding to the PD1BB switch receptor, an excitatory signaling cascade would result in T-cell activation. Both in-vitro and in-vivo, MILs containing the PD1BB switch receptor exhibited improved cytotoxicity and survival compared to wild type MILs. However, this benefit was not seen with PBLs. Nevertheless, we have shown that a PD1BB switch receptor demonstrates an improvement over WT MILs both in-vivo and in-vitro. Pursuing PD1BB switch receptor MILs as a therapeutic agent in MM is certainly worth consideration.

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INTRODUCTION

Programed death 1 (PD-1) and programmed death ligand 1 (PD-L1) have revolutionized the field of immunotherapy in solid tumors. Interaction between PD-1 and PD-L1 dampens T-cell activation and is used as an "immune checkpoint" to restrain T-cell activation under normal conditions to prevent autoimmune reactions. Tumors, through expression of PD-L1, can use this mechanism to escape immune surveillance by inducing tolerance and anergy of activated T-cells. As such, blocking the interaction between PD-1 and PD-L1 should activate the immune system and allow tumor-specific T-cells to recognize and destroy the tumor. Currently, data from multiple clinical trials confirm this.

Recently, chimeric antigen receptor (CAR) T-cell therapy has garnered a lot of attention in the field of immunotherapy, notably with the first FDA approval CAR T-cell therapy[1]. By transforming non-tumor-specific T-cells into highly specific, cytotoxic T-cells patients have seen dramatic responses in multiply relapsed disease. However, cytokine release syndrome and off-target engagement of these gene-modified T cells can have serious and even fatal effects. Additionally, inadequate CAR T-cell persistence or down-regulation of the tumor antigen may occur, rendering CAR T-cell therapy ineffective upon disease relapse.

Our lab has focused on the use of MILs (marrow infiltrating lymphocytes) as a source of T-cells to treat multiple myeloma. MILs provide an enriched population of antigen-experienced central memory T-cells, providing an effective source of cells for

immunotherapy, especially in myeloma where bone marrow is also the site of tumor. While MILs have been shown to provide a clinical benefit for numerous patients, many still eventually relapse. This may be an issue of T-cell persistence and/or T-cell anergy, which we aim to alleviate by modifying MILs with a PD-1 switch receptor.

CHAPTER ONE: PD-1 EXPRESSION IN MULTIPLE MYELOMA

1.1 PD-1 May Play a Unique Role in Multiple Myeloma

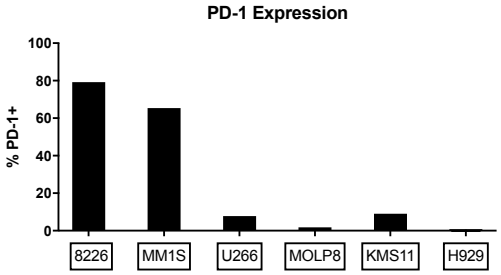
Unlike most tumors, multiple myeloma (MM) not only expresses PD-L1 but also its cognate receptor, PD-1 (Figure 1A). This feature is particularly interesting because we tend to think of PD-1 expression as exclusive to T-cells. PD-1 blockade prevents tumor-induced T-cell anergy by blocking the interaction between T-cell PD-1 and Tumor PD-L1 or PD-L2. However, in the case of MM, PD-1 blockade may affect and also enhance the tumor itself by facilitating its outgrowth. If PD-1 signaling serves a similar role in the tumor as it does in T-cells, interactions between PD-1 and PD-L1 or PD-L2 on tumor cells may serve to keep the tumor in a state of equilibrium. Blocking PD-1 on the tumor may create a more activated MM tumor and increase tumor cell proliferation. While increasing tumor cell proliferation may seem detrimental, it may also lead to increased antigen presentation, allowing more opportunities for T-cells to recognize and attack the tumor, potentially resulting in a decreased tumor burden. In this case, blocking PD-1 on the tumor as well as T-cells may result in a more "targetable" tumor as well as more activated T-cells.

1.2 PD-1 Blockade Alone Has No Effect on Myeloma Tumor Growth or Cytotoxicity

The effect of PD-1 blockade in six different myeloma cell lines with various levels of PD-1 expression was tested for up to 6 days (Figure 1A-C). No significant differences in tumor cell viability or proliferation were seen in tumor cells alone (Figure 1B-C).

Additionally, when MILs from two patients were co-cultured with each of 3 MM cell lines (8226, MM1S & U266), PD-1 blockade did not contribute to any significant differences in tumor growth compared to cells in media alone (Figure 1D).

A)



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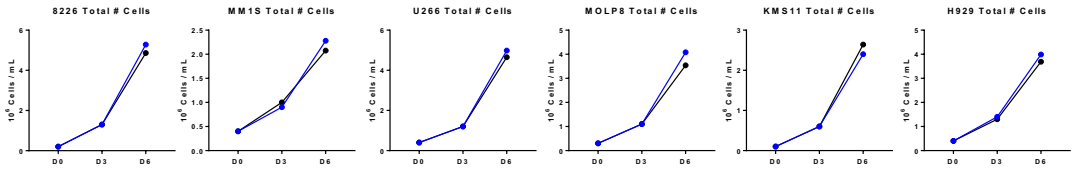

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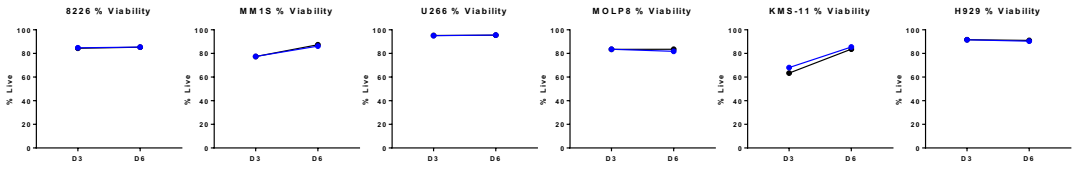

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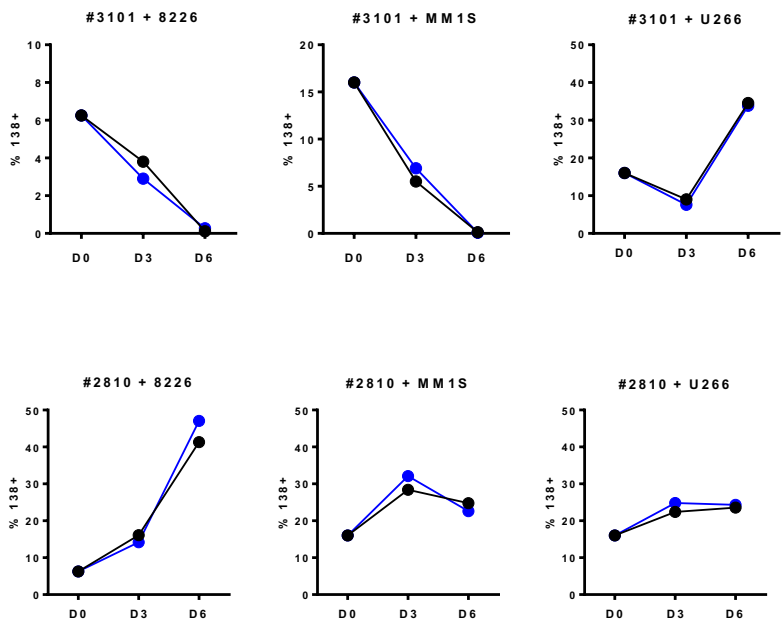


Figure 1: PD-1 is expressed in MM cell lines but PD-1 blockade has no effect on growth or viability of MM cell lines alone or in co-culture with MILs. A) Expression of PD-1 was determined in six MM cell lines by flow cytometry. **B-C)** Nivolumab was added to six MM cell lines at 10ug/mL and cells were counted using a hemocytometer and trypan blue to determine the number of cells and viability after 3 or 6 days. **D)** Three MM cell lines were co-cultured with MILs from two patients (3101 and 2810) with or without 10ug/mL nivolumab. Flow cytometry was used to determine the percentage of CD138+ cells in the co-cultures after 3 or 6 days.

This data does not support the hypothesis that PD-1 blockade increases tumor cell proliferation. However, in this model, PD-1 blockade also does not appear to increase T-

cell mediated tumor cytotoxicity. It should be noted, however, that this is a short-term experiment and the role of PD-1 blockade on T-cells as well as tumor may require more time to observe an effect and that the kinetics of tumor outgrowth of a MM cell line is significantly quicker than primary malignant myeloma.

1.3 Clinical Data of single agent Nivolumab

In concordance with the observed in-vitro data, single agent Nivolumab has shown no clinical benefit in patients with myeloma[2]. In a Phase 1b study including 27 MM patients, 17 (63%) had stable disease and only 1 patient obtained a CR, but only following radiotherapy. The remaining 9 patients had progressive disease[2]. Similar results were seen in a smaller study following four MM patients receiving single agent nivolumab (Figure 2). In this study, all four patients continued to see a rise in M-protein levels while on single agent Nivolumab and there was some concern that nivolumab could have actually increased the growth kinetics of the MM.

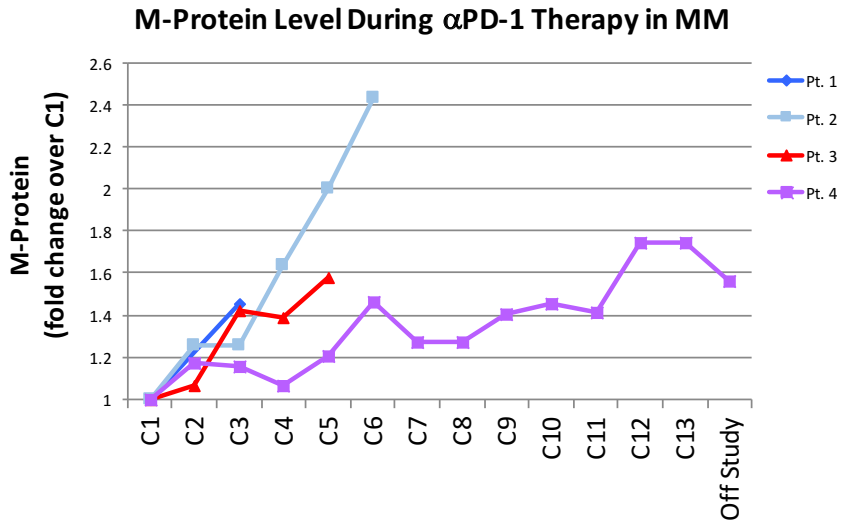


Figure 2: M-protein levels continue to increase in MM patients receiving single agent Nivolumab. M-protein levels were evaluated in four MM patients receiving single agent nivolumab.

1.4 High Levels of PD-1, PD-L1 and PD-L2 Correlate with Better Response in MM

While PD-1 blockade has been deemed ineffective in MM as a single agent, intriguing correlations have been made between survival and expression of PD-1 as well as its ligands on MM tumor cells. Previous data from our lab measured the expression of PD-1 and PD-L1 on tumor cells from 10 patients by flow cytometry prior to and up to 1 year after receiving MILs therapy (Figure 3). When patients were analyzed by clinical response, it becomes clear that there was a correlation between the levels of PD-1 and PD-L1 on tumor at baseline and clinical outcomes. Interestingly, after treatment these expression levels invert.

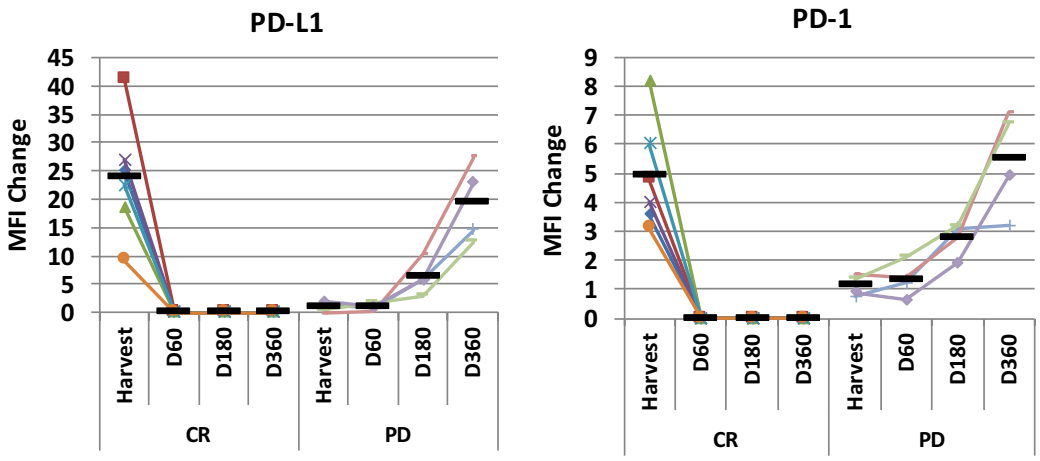


Figure 3: High expression of PD-L1 & PD-1 on primary MM tumors prior to therapy correlates with clinical response. Flow cytometry was used to measure expression of PD-L1 and PD-1 on primary MM tumors from ten patients prior to and 60, 180 and 360 days after receiving MILs. Patients were then divided based on response to MILs therapy

While data from these ten patients is certainly suggestive of a connection between high levels of PD-1 and PD-L1 on tumor and clinical response, evaluation of more patients would undoubtedly be more influential. Fortunately, CoMMpass, a study by the Multiple Myeloma Research Foundation (MMRF) includes over 1,000 patients in which bone marrow biopsies are taken from patients at the beginning of the study, at response to treatment and at relapse. RNA-seq was performed on patients' tumors and all data is available to the public. Levels of PD-1, PD-L1 and PD-L2 were used to divide patients into groups of "high expressers" and "low expressers" based on FPKM

(Fragments Per Kilobase per Million mapped reads) and overall survival (OS) and progression-free survival (PFS) were assessed (Figure 4). When examining PD-L1 & PD-L2, "high expressers" (high levels of both PD-L1 & PD-L2) showed no statistically significant differences in OS or PFS. However, when co-expression of PD-1 is taken into account along with PD-L1 & PD-L2, significant differences in both OS and PFS are seen. This data suggests that patients with tumors co-expressing high levels of PD-1 at baseline along with its ligands, PD-L1 & PD-L2, have a more favorable clinical prognosis. Conversely, low levels of or blocking PD-1 signaling may bestow a more aggressive tumor phenotype. This knowledge supports the need to specifically target T-cells when administering checkpoint inhibitor therapies in MM without affecting tumor cells.

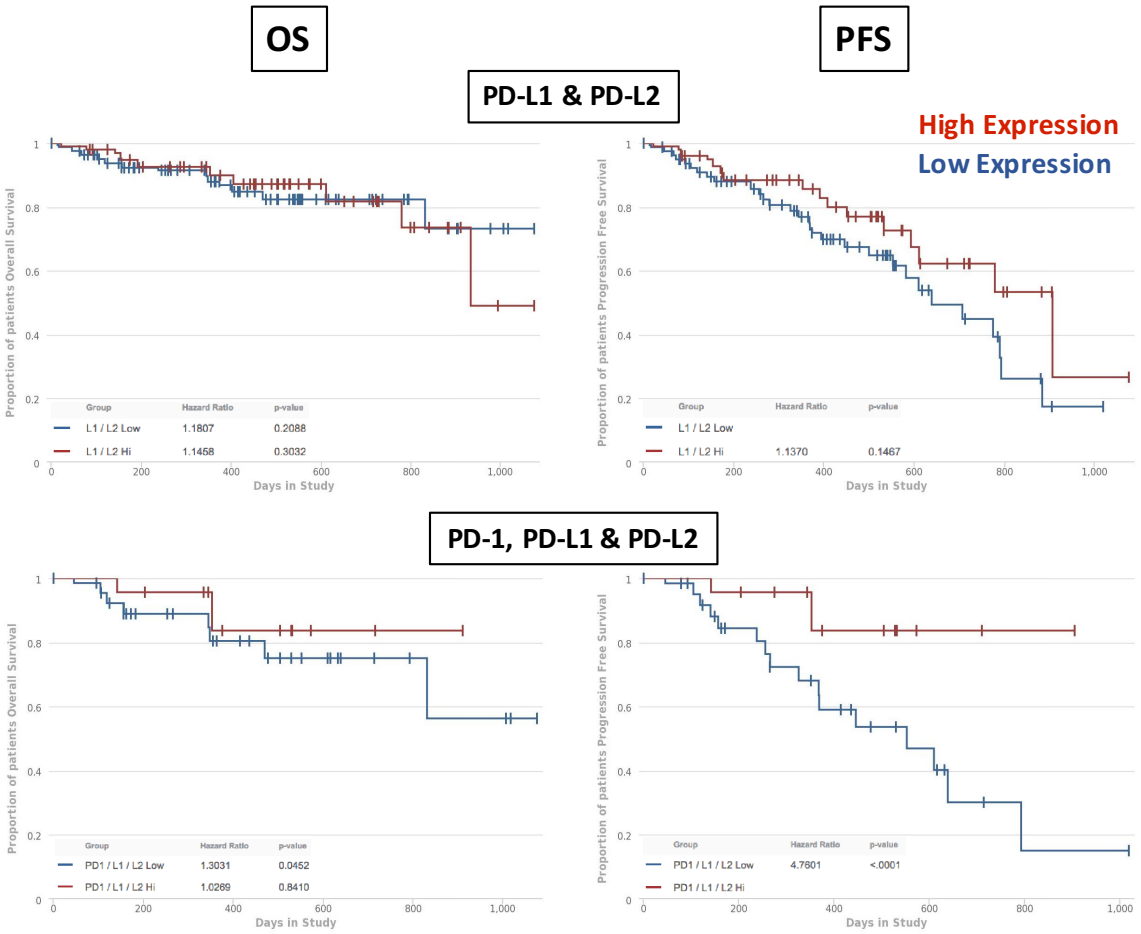


Figure 4: Co-expression of PD-1, PD-L1 and PD-L2 on MM tumors at baseline correlates with increased OS and PFS. Patients with either high or low baseline expression of both PD-L1 and PD-L2 or PD-1, PD-L1 and PD-L2 were grouped together and PFS and OS were evaluated. Analysis was performed using analysis tools and data available through the MMRF research portal.

CHAPTER TWO: CREATING A SWITCH RECEPTOR

2.1 PD-1 / 4-1BB Switch Receptor: Making a Better MIL

2.1.1 Why Use MILs?

Marrow-infiltrating lymphocytes (MILs) are bone marrow-derived T-cells that are expanded ex-vivo; T-cells are not isolated prior to expansion. Because bone marrow is the site of myeloma tumor, this means that MILs are expanded in the presence of tumor cells, increasing the likelihood of producing tumor-specific T-cells. The bone marrow is also a reservoir for central memory T-cells, which are the most efficient at maintaining long-term immunity[3,4]. MILs possess many other distinctive properties vital to effective adoptive T cell therapy: they exist in all patients, are naturally tumor-specific, traffic to the bone marrow upon reinfusion and persist over time[5]. While PBLs are more plentiful and more easily accessible, they have been shown to lack the intrinsic tumor-specificity that MILs possess[5]. MILs have been used clinically in the setting of adoptive T-cell therapy, and while patients receiving MILs have seen clinical responses[6], many patients still eventually relapse, likely due to a decline in persistence or increased exhaustion of MILs. If the survival and activation of MILs could be prolonged, consequently extending the lives of patients, this would add incredible value to MILs therapy.

2.1.2 Why Use PD-1?

While MILs are naturally tumor-specific and have shown great clinical benefit, tumor-specific anergy remains a problem limiting greater therapeutic efficacy. PD-1 is a key mediator through which tumors induce T-cell anergy and evade immune attack.

However, blocking PD-1 alone in MM has clinically shown no effect[2] and may actually be counter-productive. In part, this may be due to the fact that PD-1 is expressed on MM cells themselves and may play a role within the tumor, not just on T-Cells. While PD-1 blockade as a single agent does not work, in combination with an IMiD (immunomodulatory imide drug) such as revlimid or lenalidomide, PD-1 blockade has been shown to decrease tumor burden in patients, even in those previously refractory to IMiD therapy[7,8]. The reason for the observed decrease in tumor burden in IMiD refractory patients may be due to synergistic effects of T-cell activation from the IMiD while also preventing these activated T-cells from becoming anergic upon encounter with tumor through PD-1 blockade. Perhaps in combination, the negative effects of PD-1 blockade on the tumor are outweighed by the positive impact on T-cells. However, if PD-1 blockade could be targeted to T-cells alone in addition to activating them, perhaps even better results could be achieved.

So, rather than block PD-1 systemically, a PD-1 switch receptor was made in order to re-program the role of PD-1 within the T-cell itself by switching signaling via PD-1 from an inhibitory to an excitatory signaling cascade. Using a lentiviral vector to transduce T-cells, this PD-1 switch receptor can be expressed exclusively on T-cells. By transducing cells to express higher levels of switch receptor than naturally expressed PD-1, the switch receptor will out-compete natural PD-1 for binding to ligands, effectively blocking naturally expressed PD-1 on T-cells. Additionally, binding of ligand to the PD-1 switch receptor will not lead to T-cell inactivation, as naturally expressed PD-1 would, but instead will generate or maintain T-cell activation. Creating a MIL that

can evade tumor-induced anergy and remain or become activated while also retaining its intrinsic tumor-specificity could vastly improve MILs therapy.

2.1.3 Why use just a 4-1BB Intracellular Signaling Domain?

The design of our switch receptor includes 4-1BB alone as the intracellular signaling domain. Other switch receptors and later generation CARs use two or three intracellular signaling domains, nearly always including CD28 and/or CD3-zeta[9–12]. Both CD28 and CD3-zeta are components of the T-cell receptor and impart a strong activation signal within T-cells and high levels of stimulating cytokines such as IL-2 and IFNγ. However, one drawback to such strong T-cell activation is the possibility of over-activation and eventual anergy or even cell death. Our goal is not to make hyper-activated T-cells, but rather to generate T-cells capable of persistence and maintaining tumor-recognition without succumbing to tumor-induced anergy. There is evidence to show that signaling through 4-1BB vastly enhances T-cell persistence as well as tumor cytotoxicity in-vivo[13,14]. These effects may be due to the induction of cytotoxic CD8+ T-cell proliferation and survival[15,16] as well as up-regulation of the antiapoptotic molecule Bcl-X_L[17,18]. For these reasons, 4-1BB was chosen as the intracellular signaling domain for the PD-1 switch receptor.

2.1.4 Why Use a 4-1BB Transmembrane Domain?

The transmembrane (TM) domain, which links the extracellular PD-1 domain to the intracellular 4-1BB signaling domain, helps transmit the signal from the extracellular

domain (ECD) to the intracellular domain (ICD) and contributes to the overall activity of the switch receptor. To date, most chimeric receptors have used either a CD8 or CD28 TM domain[9–12]. Carl June's lab uses a CD8 TM domain for a majority of their receptors, while others[9,10] use a CD28 TM domain, which is the same as the most proximal ICD. Interestingly, Noessner's group compared both CD8 and CD28 TM domains in the context of a PD-1_CD28 switch receptor[12], and showed that using the same TM domain as the most proximal ICD imparted higher expression and better signaling than a different (CD8) domain. Therefore, we decided to include the transmembrane domain of 4-1BB itself instead of a TM domain unrelated to the ICD in order to maintain the integrity of the entire portion of 4-1BB responsible for transmitting its signal.

2.1.5 Why Use an EF1a Promoter?

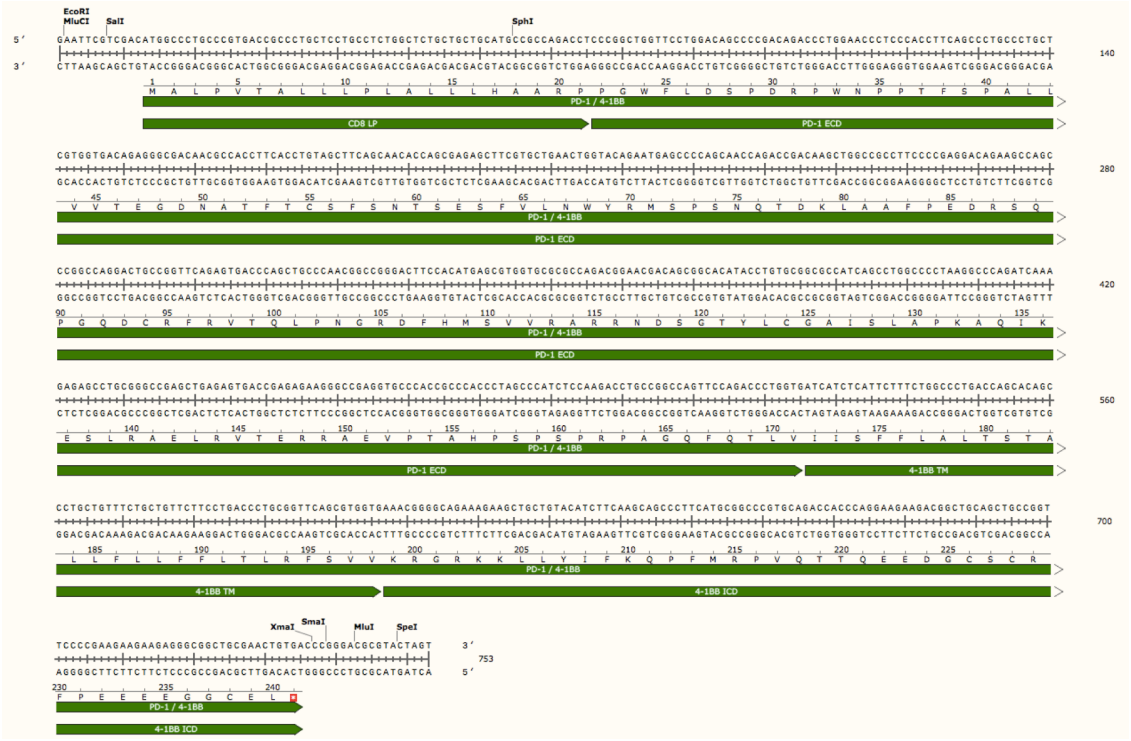
Both the CMV and EF1a promoters are commonly used. The EF1a promoter was chosen over a CMV promoter due to its more consistent expression across multiple cell types as well as stability in expression in cells with varying states of activation. While both are strong promoters, the use of a CMV promoter results in widely variable expression across different cell types and can also results in variable expression depending on the activation state of a cell, whereas EF1a has more stable expression[19,20].

2.2 Making a PD-1 / 4-1BB Switch Receptor

2.2.1 Sequences

To make the PD-1 / 4-1BB (PD1BB) switch receptor, the amino acid sequences of the CD8 LP (AA 1-21), PD-1 ECD (AA 21-170) and 4-1BB TM and ICD (AA 187-255) were found using Uniprot.com. Next, the amino acid sequences were reverse-translated into DNA sequences using Bioinformatics.org. We then added several restriction sites at both the beginning and end of the construct as well as stop and start codons at the respective ends (Figure 5A-B). The final sequence was then given to GeneArt to synthesize. Additionally, GeneArt cloned the synthesized construct into a vector purchased from Clontech (Figure 5C-D) (pLVX-EF1alpha-IRES-mCherry Vector, catalog# 361987).

A)



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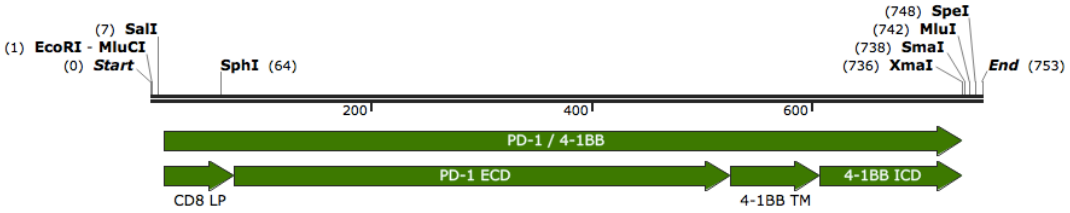
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(1) EcoRI - MluCI
(0) Start
SphI (64)
(748) SpeI
(742) MluI
(738) SmaI
(736) XmaI
End (753)
200
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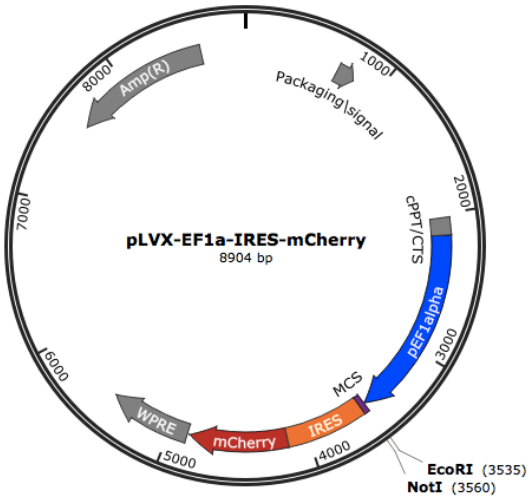
600
PD-1 / 4-1BB
PD-1 ECD
4-1BB ICD
CD8 LP
4-1BB TM

C)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NotI (3560)

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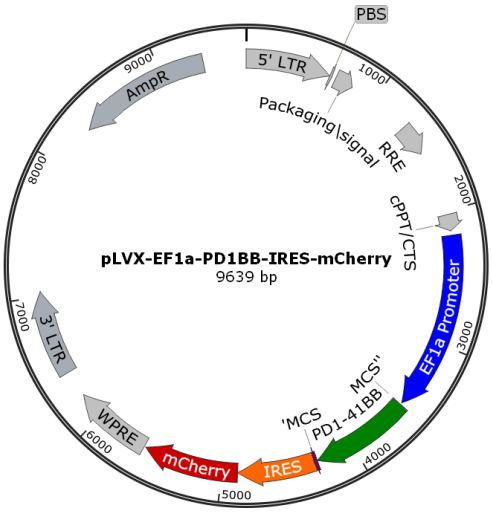
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'MCS
PD1-41BB
IRES
mCherry
WPRE
3' LTR

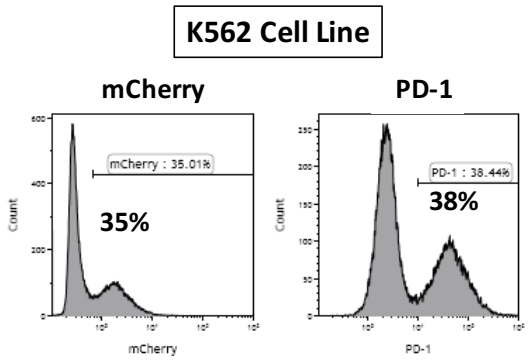
Figure 5: Sequence & Structure of the PD1BB switch receptor and structure of pLVX-EF1a-IRES-mCherry vector used to express the switch receptor. A) Sequence of the PD-1 / 4-1BB switch receptor including restriction sites added to either end. **B)** General structure of the PD1BB switch receptor. **C-D)** Structure of the pLVX-EF1a-IRES-mCherry vector without and with the PD1BB switch receptor inserted

2.2.2 1st Attempt in an mCherry Vector

The PD1BB switch receptor was first inserted into a vector containing mCherry (Figure 5C-D). mCherry was chosen as a fluorescent marker because not only could it be detected by flow cytometry, a key tool used by our lab, it would also be incredibly advantageous when performing in-vivo experiments to track the location and relative quantity of transduced T-cells.

However, while successful transduction was achieved in several tumor cell lines (Figure 6A), after numerous attempts to transduce T-cells, successful transduction was never accomplished in T-cells without also killing the cells (Figure 6B). Having deduced that the switch receptor sequence was correct due to successful expression in cell lines, it was hypothesized that perhaps something about the vector itself was contributing to the lack of expression in viable T-cells. The decision was made to cut the switch receptor sequence out of the mCherry vector and insert it into a new vector which expressed GFP.

A.



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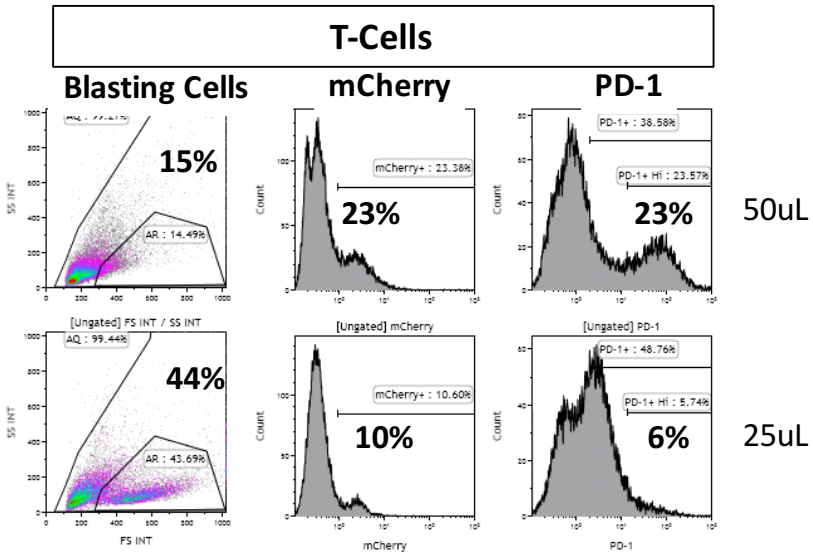


Figure 6: Transduction of cells with pLVX-EF1a-PD1BB-IRES-mCherry vector was successful in cell lines but not T-cells. A) The K526 cell line and **B)** T-cells were transduced with pLVX-EF1a-PD1BB-IRES-mCherry. After 5 days, expression of mCherry and PD-1 was determined by flow cytometry.

2.2.3 2nd attempt in a GFP Vector

PCR was used to remove the PD1BB switch receptor from the pLVX-EF1a-IRES-mCherry vector using forward and reverse primers (Figure 7A-B). EcoRI & NotI restriction sites were added by incorporating them into the primers.

A)

PD1BB + EcoRI Fwd: 5' AT**GAATTC**ATGGCCCTGCCCGTGA

EcoRI

B)

PD1BB + NotI Rev: 5' AT**GCGGCCGC**CAGTTCGCAGCCGCCC

NotI

Figure 7: Forward and Reverse Primers used to remove the PD-1 / 4-1BB switch receptor from the pLVX-EF1a-IRES-mCherry vector. A) Forward primer with EcoRI. **B)** Reverse primer with NotI

The PCR product was run on a gel 1.2% acrylamide gel and the appropriate band for the PCR product (738bp) was cut out. DNA was extracted from the band using a Qiagen DNA Gel Extraction kit per the manufacturer's instructions and quantified using the nanodrop.

Both the switch receptor insert DNA & new pCDH-EF1-MCS-T2A-copGFP plasmid DNA (Systembio, catalog #CD526A) (Figure 8A) were cut with the EcoRI and NotI

restriction enzymes. A gel was run with both the cut and un-cut pCDH-EF1-MCS-T2A-copGFP backbone DNA and the band for cut backbone was removed from the gel and the DNA was purified using the Qiagen DNA Gel Extraction kit.

The cut PD1BB switch receptor PCR insert was ligated into cut backbone using DNA T4 ligase (Figure 8B), transformed into competent DH5a cells by heat-shock and plated on LB agar plates overnight. Colonies were picked from the plates and grown in 4mL LB+Amp broth overnight. MiniPreps of cultured colonies were performed using the Qiagen DNA MiniPrep kit per the manufacturer's instructions. DNA from the minipreps were digested with the SphI restriction enzyme and run on a 1.2% acrylamide gel to verify that the switch receptor was inserted into the plasmid. MiniPreps with bands indicating that the switch receptor had been inserted were identified and sent for sanger sequencing to the core at Johns Hopkins.

After the correct switch receptor sequence was confirmed, 1mL of transformed DH5a corresponding to the verified miniprep was used to seed a larger 500mL batch of bacteria which was then grown overnight. Following overnight culture, the E. Coli were pelted by centrifugation at 3,000 rpm for 30min @ 4^{o}C. MaxiPreps were then performed using the Qiagen kit per the manufacturer's instructions. The DNA was then quantified using a nanodrop and frozen at -20^{o}C.

A) **B)**

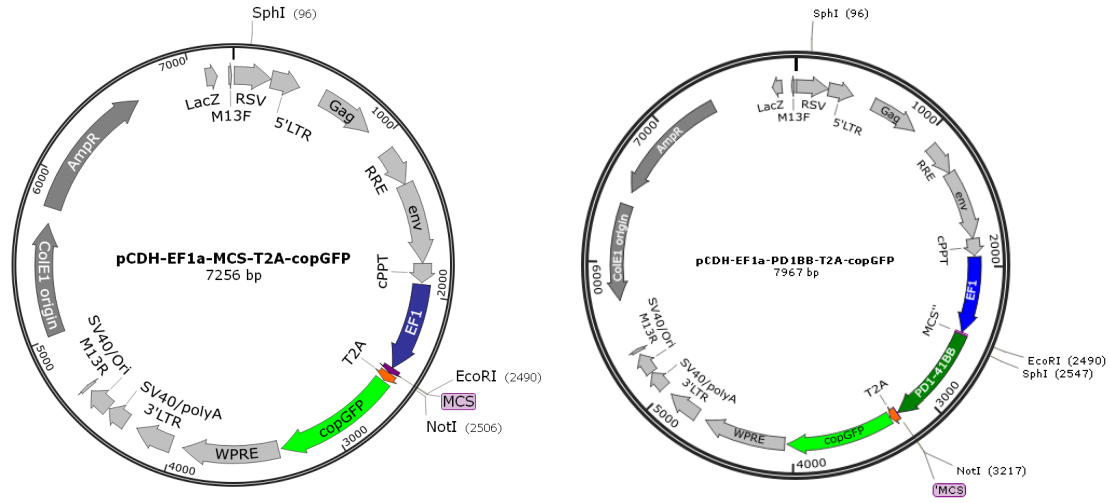


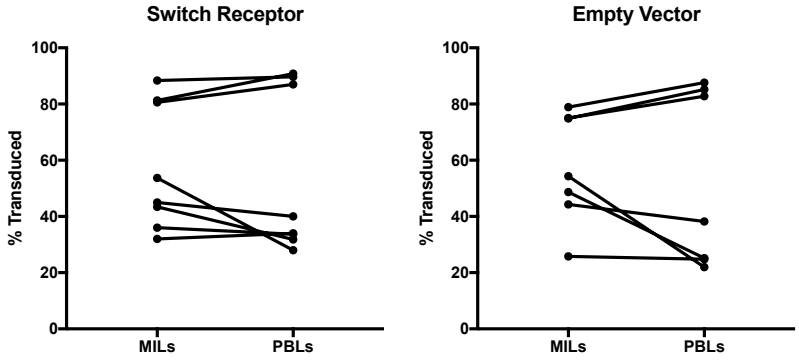
Figure 8: The PD1BB switch receptor was inserted into the pCDH-EF1-MCS-T2A-copGFP plasmid. A) pCDH-EF1-MCS-T2A-copGFP plasmid backbone (empty vector) **B)** PD1BB switch receptor inserted into the pCDH-EF1-MCS-T2A-copGFP

CHAPTER THREE: BIOLOGY OF THE SWITCH RECEPTOR

3.1 The PD1BB Switch Receptor is Easily and Highly Expressed in MILs and PBLs

The PD1BB switch receptor is easily transduced into both MILs and PBLs. This is done using concentrated lentivirus without the use of chemicals, such as polybrene and without spinfection, which cannot be done in the presence of magnetic CD3/CD28 beads without killing the cells. The PD1BB switch receptor is able to transduce between 28-90% of T-cells in both MILs and PBLs (Figure 9A) and similar transduction efficiencies are seen with the PD1BB switch receptor and the empty vector (EV) (Figure 9B). The levels of transduction can be slightly variable from patient to patient and between MILs and PBLs of the same patient (Figure 9A). Additionally, the amount of transduction can be adjusted varying the amount of virus added. Because we transduce T-cells 2-3 days after activation and do not isolate T-cells prior to transduction, it should be noted that the population of cells is not a pure T-cell population. However, upon harvest 7-10 days post-activation, nearly 100% of the cells are CD3+.

A)



B)

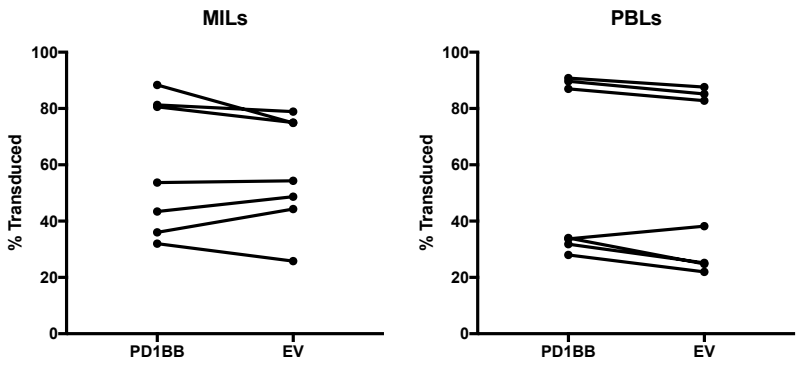


Figure 9: Transduction efficiency of the PD1BB switch receptor and empty vector in MILs and PBLs. Flow cytometry for GFP+ cells was used to determine **A)** Transduction efficiency of the PD1BB switch receptor or empty vector (pCDH-EF1a-MCS-T2A-copGFP) compared between MILs or PBLs of the same patients. **B)** Transduction efficiency of MILs or PBLs from the same patient compared between the PD1BB switch receptor or empty vector (pCDH-EF1a-MCS-T2A-copGFP).

3.2 No Differences in Growth Kinetics are Observed with the PD1BB Switch Receptor

No significant differences in growth kinetics have been observed between PD1BB switch receptor, empty vector-transduced T-cells and non-transduced T-cells (Figure 10). This is an important observation because we do not want switch receptor signaling to occur without binding to its intended ligand which could lead to activation-induced cell death. Interestingly, increased activation and growth was seen when briefly working with another construct (data not shown). This construct included extracellular CD8 and both CD3ζ and 4-1BB intracellular signaling domains. It is unclear as to why this other

construct imparted such a drastic increase in growth kinetics – perhaps it is due to strong signaling from CD3ζ which is highly employed during T-cell activation forming heterodimers with the transduced CD3ζ further amplifying TCR signaling, a mechanism that has been suggested by other studies[21].

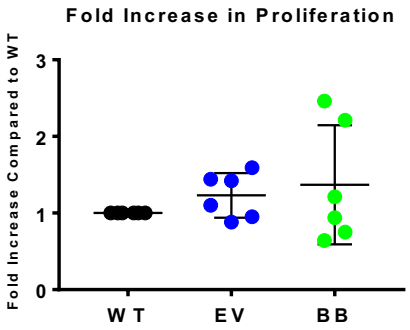
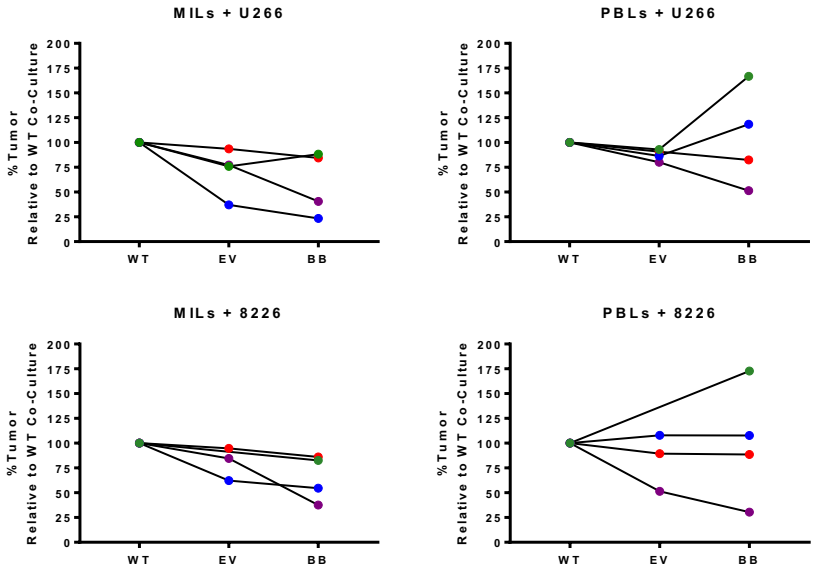


Figure 10: Differences in proliferation of wild type, empty vector and PD1BB switch receptor T-cells. T-cells that were no transduced, transduced with the empty vector (EV) or with the PD1BB switch receptor (BB) were counted upon harvesting the T-cells after 7-10 days in culture. Cell counts of WT, EV and BB T-cells were all then normalized to the number of WT T-cells to determine the fold increase (or decrease) in proliferation.

3.3 In-Vitro Tumor Cytotoxicity

In order to determine if the PD1BB switch receptor effects tumor cytotoxicity, T-cells were co-cultured with HLA-A2 matched tumor cells in-vitro. The PD1BB switch receptor MILs consistently kill more tumor than WT MILs with each of two different tumor cell lines in-vitro (Figure 11A). In contrast, PD1BB switch receptor PBLs demonstrate variable results with some killing more and others less or equivalently to WT PBLs. Surprisingly, in several patients, transduction of T-cells with the empty vector alone results in similar anti-tumor activity as those transduced with the PD1BB switch receptor.

A)



B)

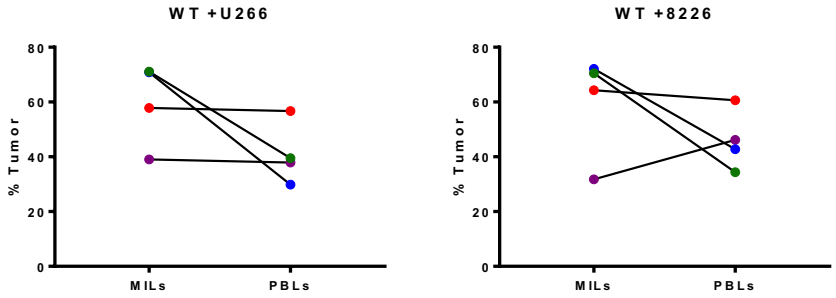


Figure 11: Tumor cytotoxicity is consistently increased by the PD1BB switch receptor in MILs but not PBLs, however WT MILs do not demonstrate more tumor cytotoxicity than WT PBLs. MILs or PBLs what were not transduced (WT), transduced with the empty vector (EV), or with the PD1BB switch receptor (BB) were co-cultured with 8226 or U266 tumor cell lines for several days and the percentage of CD138+ tumor cells was determined by flow cytometry. **A)** For each patient, the percentage of CD138+ tumor cells was normalized to that of the WT T-cells. **B)** For each patient, the percentage of CD138+ tumor cells was compared between MILs and PBLs for each of the two tumor cell line co-cultures.

Another surprising finding is that when observing WT T-cells in co-culture with tumor cell lines, PBLs are almost always as efficient if not more efficient than MILs at killing tumor in-vitro (Figure 11B). This data diverges from previously published data from our lab showing that MILs are more cytotoxic than PBLs[5]. However, it should be

noted that the current data is only using myeloma tumor cell lines whereas the previously published data used both cell lines as well as autologous tumor. When using tumor cell lines, even though they are HLA-A2 matched with the patient samples used, there will inherently be some degree of alloreactivity. One possibility is that PBLs are more alloreactive than MILs and that this is a contributing factor to the observed increase in anti-tumor cytotoxicity of PBLs. It has also been shown that although both MILs and PBLs show Vβ skewing prior to activation, un-activated MILs showed more baseline skewing than PBLs and upon activation and MILs maintained oligoclonality whereas activated PBLs normalized their Vβ repertoire[5]. While this may suggest that activated MILs are enriched for tumor-specific T-cells, it may alternatively suggest that MILs have a more limited range of antigen specificity than PBLs that may explain the reduced allo-reactivity.

3.4 Expression of Activation and Checkpoint Proteins: In-Vitro Co-Culture

Activation (CD69, CD25, CD28 & Granzyme B) and checkpoint markers (Lag3, Tim3, OX-40, CTLA-4, ICOS & PD-1) were examined on T-cells. Co-culture with tumor for 2 to 4 days failed to alter the expression of most of these markers compared to T-cells alone at the same time points. The one exception was ICOS, which was up-regulated on CD4+ T-cells but down-regulated on CD8+ T-cells when tumor cells were present (Figure 12). No appreciable differences were observed between WT, EV or switch receptor T-cells.

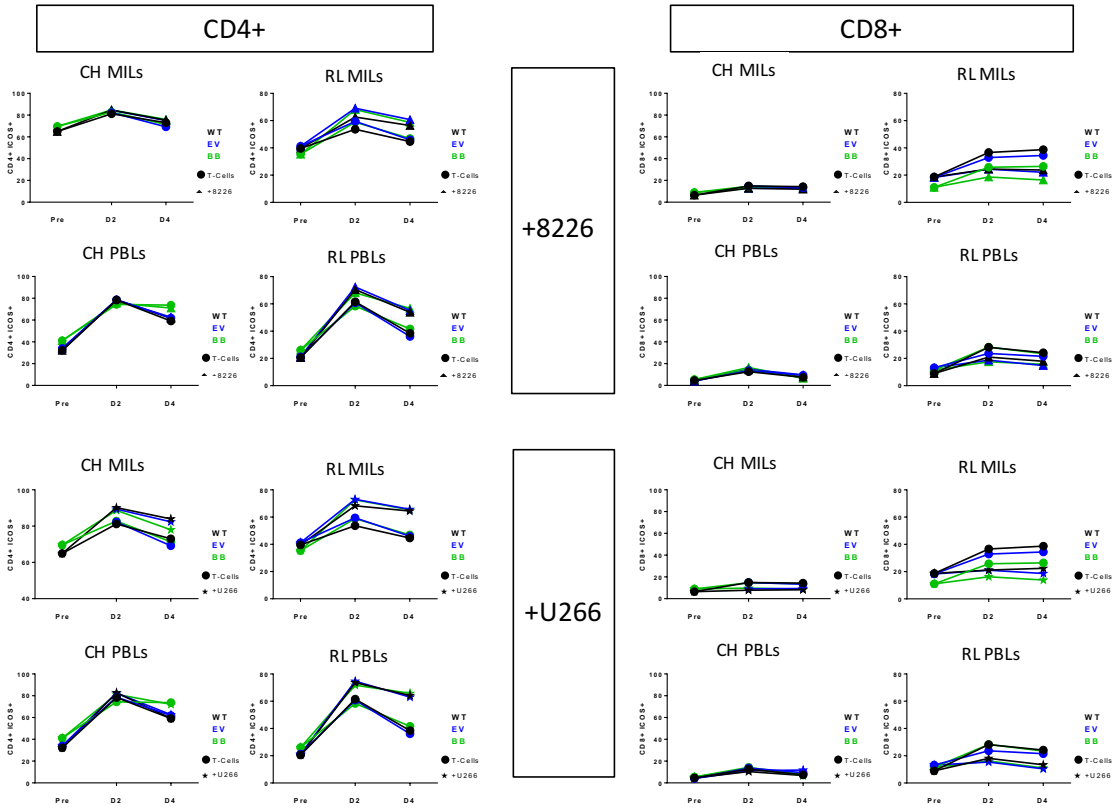


Figure 12: ICOS Expression increases in CD4+ T-cells but decreases in CD8+ T-cells upon exposure to tumor antigen. Flow cytometry was used to determine the expression of ICOS on CD4+ or CD8+ T-cells either alone (●) or in co-culture with 8226 (▲) or U266 (★).

As expected, PD-L1 on tumor cells was up-regulated in the presence of T-cells (Figure 13). PD-L1 has been shown to be up-regulated by IFNγ which is produced by activated T-cells[22,23]. While elevated PD-L1 expression was observed in the presence of T-cells, no differences were observed between MILs or PBLs or WT, EV or switch

receptor T-cells and the levels of PD-L1 on tumor. It is possible that the cell lines each have a maximum level of PD-L1 expression that can be achieved, after which no additional expression can be seen despite varying levels of T-cell IFNγ production as described below.

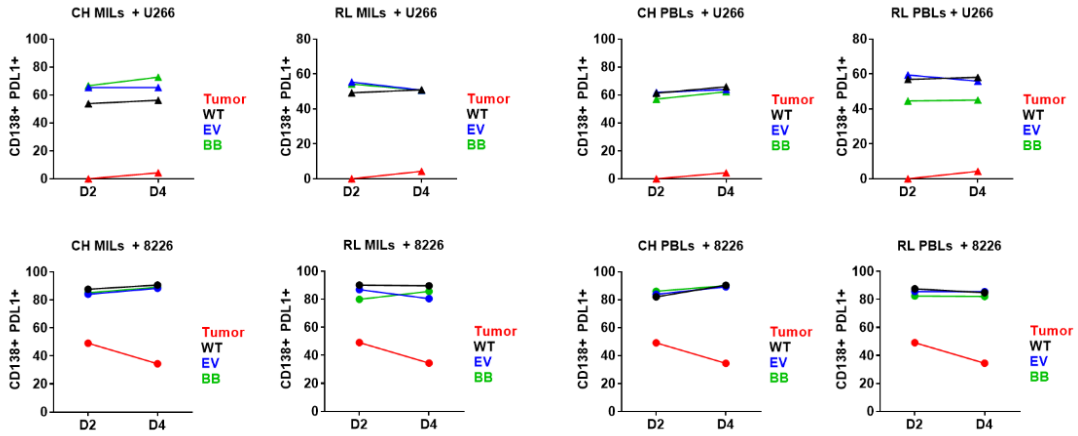


Figure 13: PD-L1 is up-regulated on tumor cells when co-cultured with T-cells. Flow cytometry was used to determine the expression of PD-L1 on U266 or 8226 tumor cell lines either alone (red) or in co-culture with wild type (black) empty vector (blue) or switch receptor (green) MILs or PBLs.

3.5 IFNγ Expression: In-Vitro Co-Culture

During T-cell co-culture with tumor cell lines, supernatants were taken at 12 and 30hrs to look at IFNγ production. When looking at T-cells alone (without the presence of tumor), IFNγ levels were much higher in MILs than PBLs at both time points (Figure 14A). Interestingly, slightly decreased levels of IFNγ were observed in all PD1BB switch

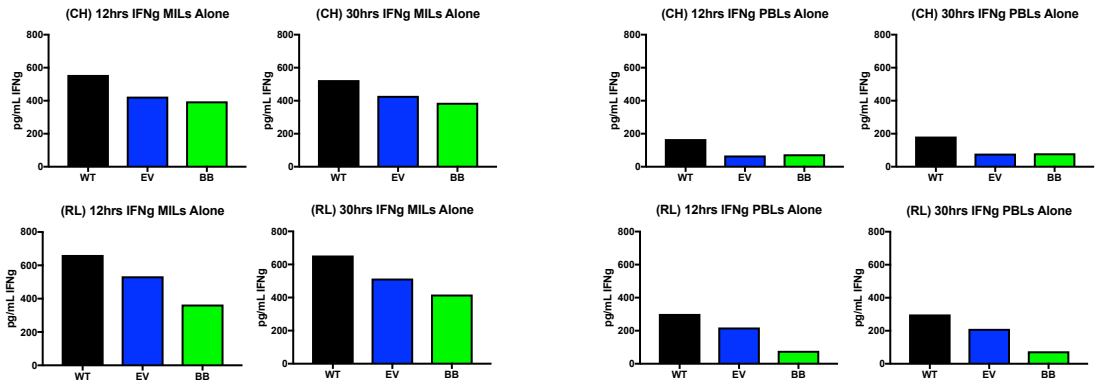
receptor T-cells. Upon encounter with U266 tumor cells, a majority of MILs as well as PBLs from both patients increased IFNγ production, indicating that these T-cells are mounting an anti-tumor response (Figure 14B). However, very little, if any increases in IFNγ production were observed in response to the 8226 tumor cell line in either patient (Figure 14C). These differences in response to cell lines may be due to the number of tumor antigens recognized by the T-cells. It is possible that the U266 cell line has more tumor antigens that are able to elicit a T-cell response than the 8226 cell line.

While production of IFNγ by T-cells alone appears to have no effect on their capacity to generate an anti-tumor response, increases in IFNγ production above amounts produced by non-tumor stimulated T-cells appear to correlate with tumor cytotoxicity (Figures 14D-E). This observation is consistent with the fact that activated T-cells produce higher levels of IFNγ than resting T-cells. Thus, the stronger the anti-tumor T-cell response is, the more IFNγ will be produced by a cell. Conversely, if little or even less IFNγ is produced in the presence of tumor when compared to T-cells alone, this likely indicative of an anergic T-cell, as observed with the MILs of patient RL with 8226 tumor.

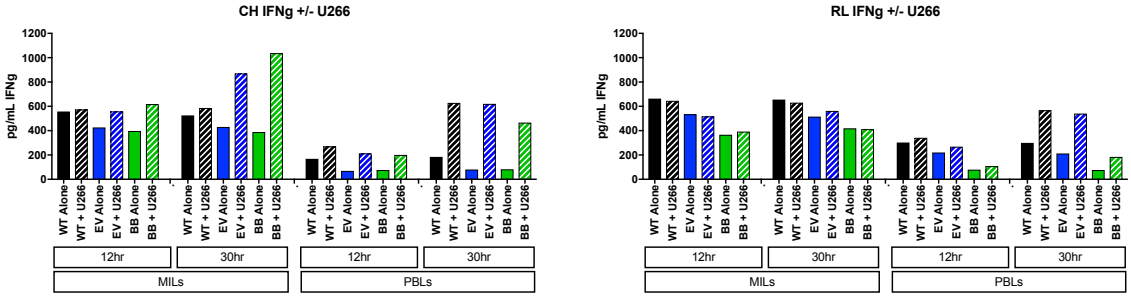
The PD1BB switch receptor MILs generated a much larger increase in IFNγ production compared to WT MILs of patient CH in the presence of U266 tumor as well as less of a decrease in IFNγ production in the presence of 8226 tumor cells (Figure 14D-E). Slightly less of a decrease was seen with the MILs of patient RL in the presence of U266 tumor, but not 8226. While only from two patients, the data suggests that the

PD1BB switch receptor confers a heightened tumor specificity of MILs and potentially evades tumor-induced anergy. However, the same does not appear to be true of PD1BB switch receptor in PBLs, in which the switch receptor has no effect (patient CH) or actually results in a decrease in IFNγ production compared to WT PBLs (patient RL). The differences in tumor-induced IFNγ production observed between PD1BB switch receptor MILs and PBLs potentially underscores the inherent tumor specificity of MILs. Assuming that MILs from these patients are more tumor specific than PBLs, the PD1BB switch receptor should help to increase T-cell activation and/or prevent T-cell anergy. It is this inherent endogenous tumor specificity of MILs that justified the rationale for developing a switch receptor approach in which we believe could only have a therapeutic benefit in MILs.

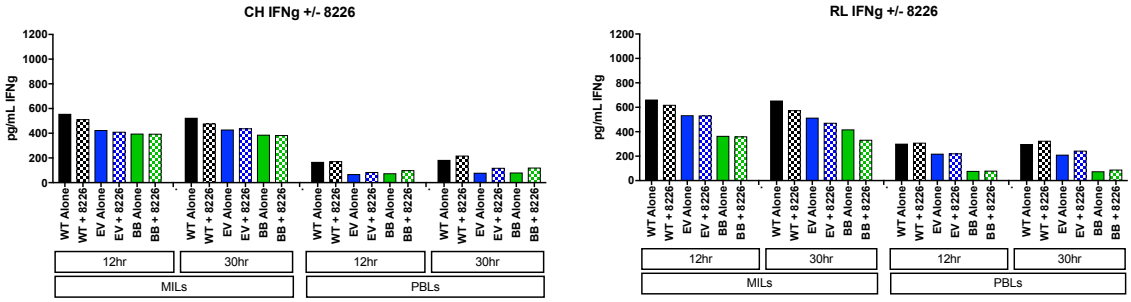
A)



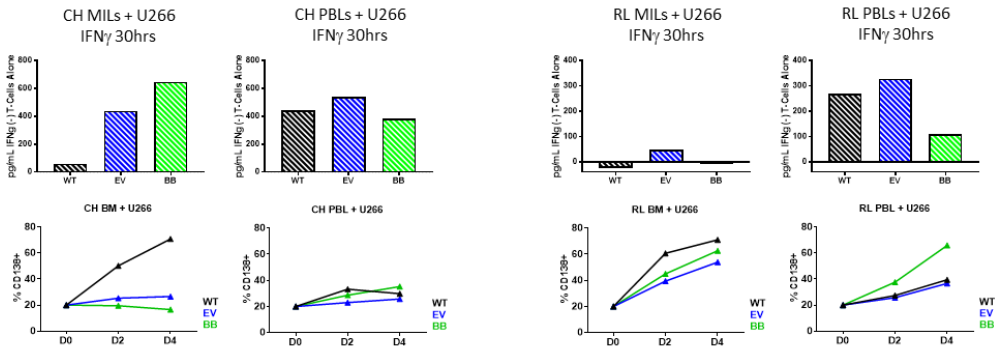
B)


CH IFNg +/- U266
RL IFNg +/- U266
pg/mL IFNg
1200
1000
800
600
400
200
0
WT Alone
WT + U266
EV Alone
EV + U266
BB Alone
BB + U266
12hr
30hr
MILs
PBLs

C)


CH IFNg +/- 8226
RL IFNg +/- 8226
pg/mL IFNg
1200
1000
800
600
400
200
0
WT Alone
WT + 8226
EV Alone
EV + 8226
BB Alone
BB + 8226
12hr
30hr
MILs
PBLs

D)


CH MILs + U266
IFNγ 30hrs
CH PBLs + U266
IFNγ 30hrs
RL MILs + U266
IFNγ 30hrs
RL PBLs + U266
IFNγ 30hrs
pg/mL IFNg(-) T-Cells Alone
WT
EV
BB
CH BM + U266
CH PBL + U266
RL BM + U266
RL PBL + U266
% CD138+
D0
D2
D4

E)

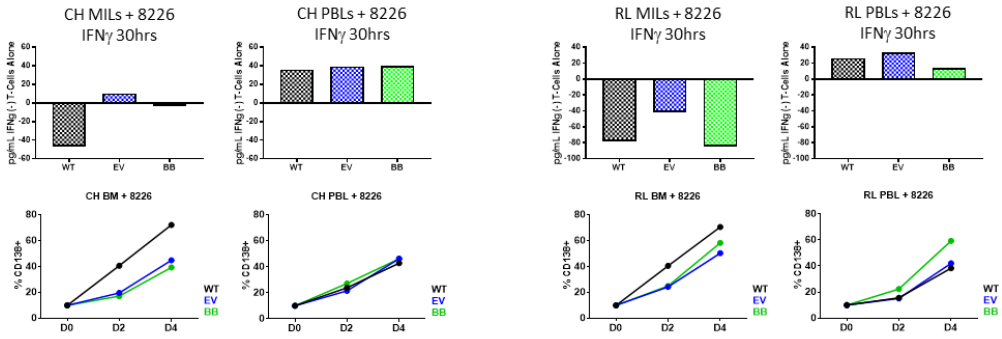


Figure 14: Increases in IFNγ production in MILs or PBLs in co-culture with tumor cell lines correlates with tumor cytotoxicity. Supernatants from cell cultures were taken after 12hrs or 30hrs in co-culture with tumor cell lines or cultured alone and used to perform an IFNg ELISA. **A)** IFNγ concentrations of WT, EV or PD1BB switch receptor MILs or PBLs cultured without tumor cell lines. **B)** IFNγ concentration of MILs and PBLs cultured alone (solid bars) or with U266 tumor cells (striped bars) or with **C)** 8266 tumor cells (hatched bars). **D)** Concentrations of IFNγ from WT, EV or PD1BB switch receptor T-cells alone were subtracted from the concentrations of those in co-culture with tumor cells (bar graphs) and compared to the percentage of CD138+ tumor cells, determined by flow cytometry, during co-culture (line graphs) with U266 tumor cells or **E)** 8226 tumor cells.

3.6 In-Vivo Methods

To test the ability of the PD1BB switch receptor to augment T-cell tumor cytotoxicity in-vivo, NSG mice were used and injected with HLA-A2 matched human MM tumor cells and activated T-cells. One day prior to injecting tumor cells, the mice were irradiated with 220 rads using an X-ray irradiation (Figure 15). The next day, $2x10^6$ U266 cells re-suspended in PBS were injected IV via the tail vein. We then began monitoring for tumor burden by measuring serum IgE levels through weekly tail-bleeding of the mice. T cells were administered the to the mice 18 days after tumor challenge. They were given activated wild type, empty vector or PD1BB switch receptor-transduced MILs or PBLs from each of 2 patients via tail vein injection with 5 mice per group. Additionally, a group of 10 mice were not given any T-cells. Following injection of T-cells, all mice were tail bled weekly to assess disease burden in the serum by IgE ELISA. Mice that became paralyzed, a consequence of high MM tumor burden in mice, or were otherwise too sick, were sacrificed. The bone marrow of sacrificed mice was harvested, counted, and analyzed by flow cytometry for CD3, CD8 and CD138 as well as T-cell memory phenotypes (CD45RO and CD62L) as well as expression of PD-1 and PD-L1 on T-cells and tumor, respectively.

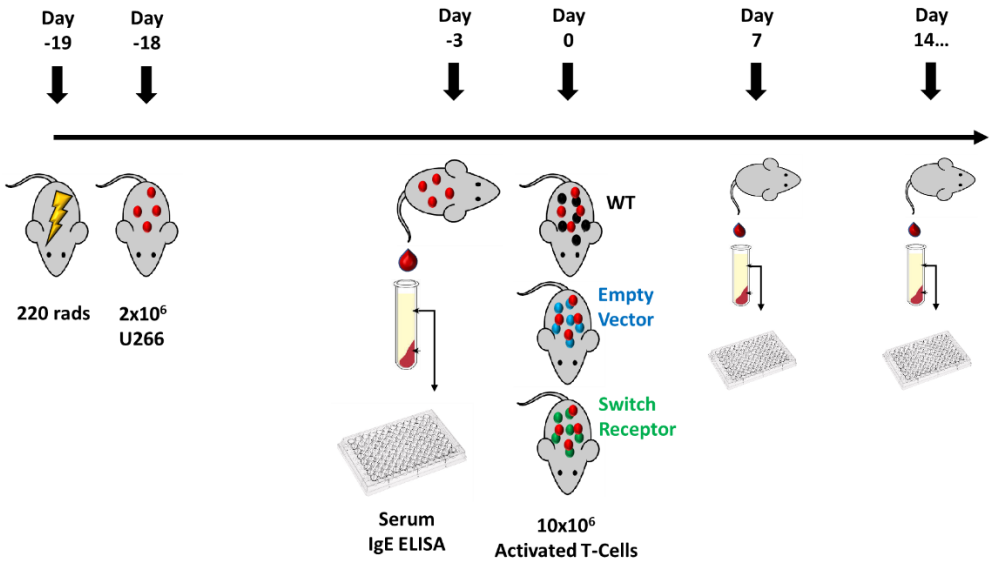


Figure 15: Timeline and schematic of in-vivo experimental procedure. 65 NSG mice were irradiated with 220 rads using x-ray irradiation 24hrs prior to tumor injection. 15 days later, mice were tail bled to collect serum and IgE ELISAs were performed to determine tumor presence and burden. 18 days after tumor injection, no T-cells or WT, EV or PD1BB switch receptor MILs or PBLs from each of two patients were injected into the mice (5 mice per group). Tail bleeds to collect serum were performed weekly and used to perform IgE ELISAs. Animals were sacrificed upon hind leg paralysis or if they were getting too sick.

The T-cells used for this in-vivo experiment were both HLA-A2+ and matched the U266 tumor at either the Cw7 or Cw3 locus as well. Whole bone marrow or PBLs were plated at $1x10^6$ cells/mL in 200ul/well in U-bottom 96-well plates. Cells were stimulated

using 2uL/10^6 cells of a CD2/CD3/CD28 tetramer (catalog #10970, Stemcell Technologies) and 200 U/mL of IL-2. Three days after stimulation, cells were transduced with either 5ul/well of the PD1BB switch receptor, 1uL/well of the empty vector or with no virus at all. Cells were grown in 96-well U-bottom plates for 7 days and then rested (cells were not split or given additional IL-2) for 3 days prior to injection into mice.

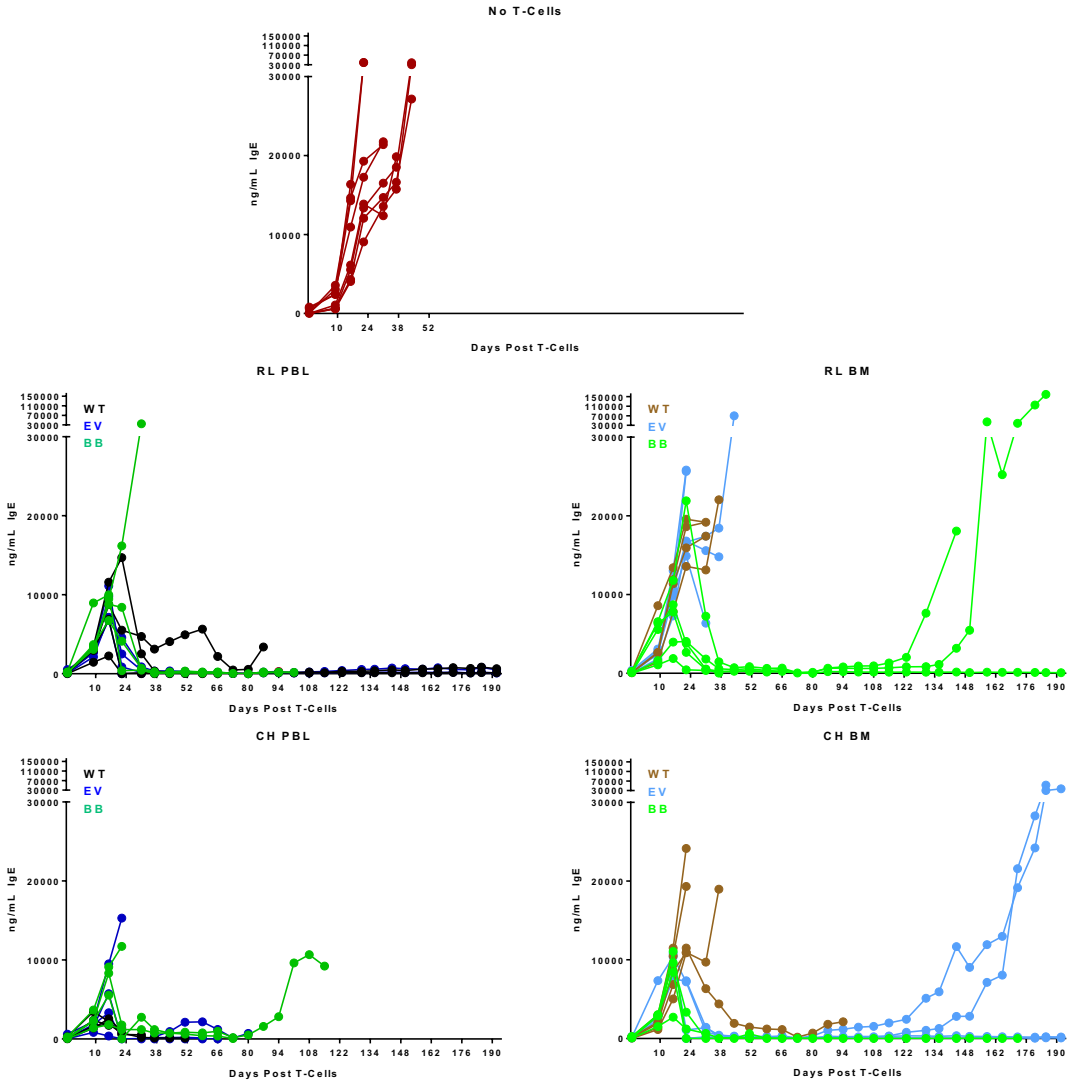
3.7 In-Vivo Cytotoxicity & Survival

Mice that did not receive T-cells all died within 62 days of receiving tumor with an average lifespan of 52 days after tumor injection (Figure 16A-C). In contrast, all mice that received any type of T-cells lived an average of 112 days after tumor injection while mice receiving WT T-cells lived an average of 89 days indicating that in general, T-cells have anti-tumor capabilities. Surprisingly, WT PBLs of both patients demonstrated rapid tumor clearance in-vivo (Figure 16A-B). Both the empty vector and PD1BB switch receptor PBLs demonstrated similar capacities to clear tumor in-vivo with both patients as well. Additionally, mice receiving the PD1BB switch receptor PBLs did not demonstrate a survival benefit compared to those receiving WT PBLs (Figure 16C), and actually decreased survival in mice receiving the PD1BB switch receptor PBLs from patient RL. This data is on one hand seemingly at odds with the enhanced tumor specificity observed in the wild type (non-transduced) but consistent with our hypothesis that the intrinsic nature of MILs (as compared to PBLs) should significantly enhance the anti-tumor efficacy with transduction with a switch receptor.

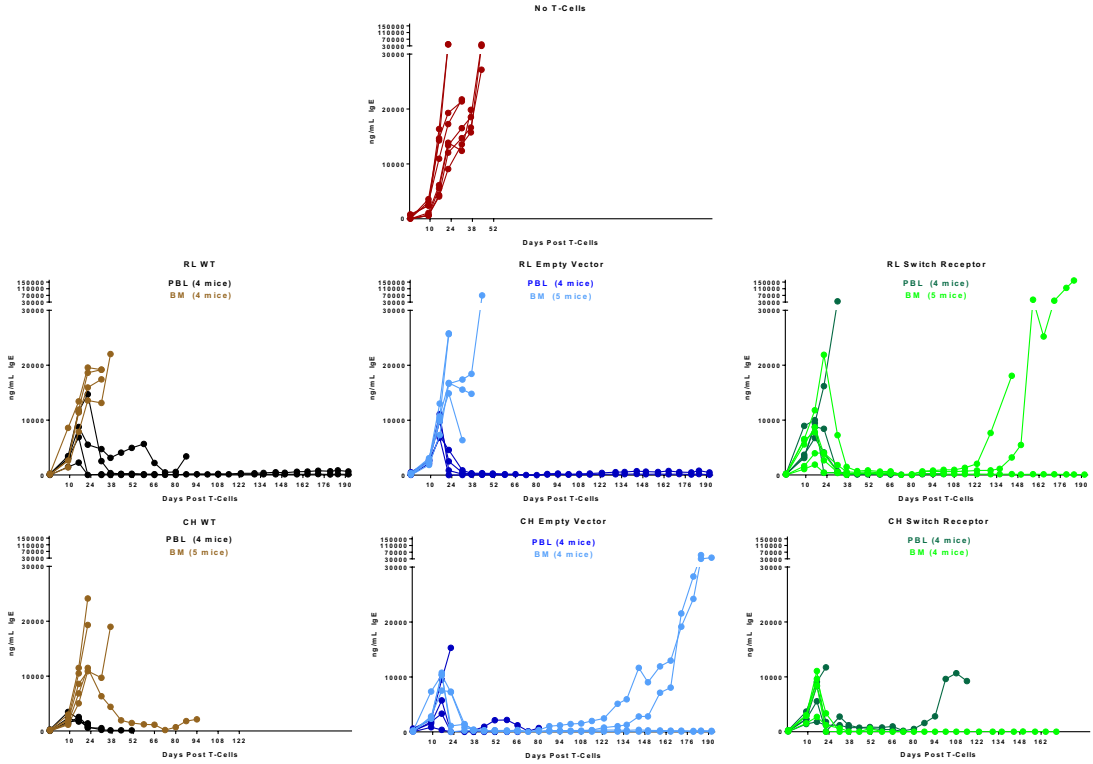
In contrast, mice receiving WT MILs from both patients were largely unable to clear tumor and did not demonstrate much, if any, survival benefit compared to mice that did not receive any T-cells (Figure 16B). However, the PD1BB switch receptor MILs from both patients demonstrated improved tumor cytotoxicity in-vivo compared to WT MILs, and radically so in patient RL. This data, much like the in-vitro data, demonstrates that the PD1BB switch receptor improves the anti-tumor cytotoxicity of MILs as well as survival. Interestingly, the empty vector MILs of patient CH, but not RL, appear to improve the ability of MILs to clear tumor in-vivo and demonstrate a survival benefit, even compared to the PD1BB switch receptor. It may be that the virally transducing a T-cell can create a more persistently activated T-cell, resulting in longer term survival. However, why the empty vector would cause similar tumor cytotoxicity and in-vivo survival as the PD1BB switch receptor is unclear.

Upon analyzing the T-cell phenotypes in the bone marrow of the mice, a correlation between the number of CD8+ central memory (CD45RO+ CD62L+) T-cells and survival became apparent (Figure 16D). The more CD8+ central memory cells found in the bone marrow, the longer the mice survived. This data is not surprising given the known anti-tumor capabilities of CD8+ central memory T-cells[24,25]. This knowledge could potentially be used to select for sub-types of T-cells (like CD8+ central memory cells) that may confer even greater anti-tumor properties as well as in-vivo longevity than the infusion of all expanded T-cells.

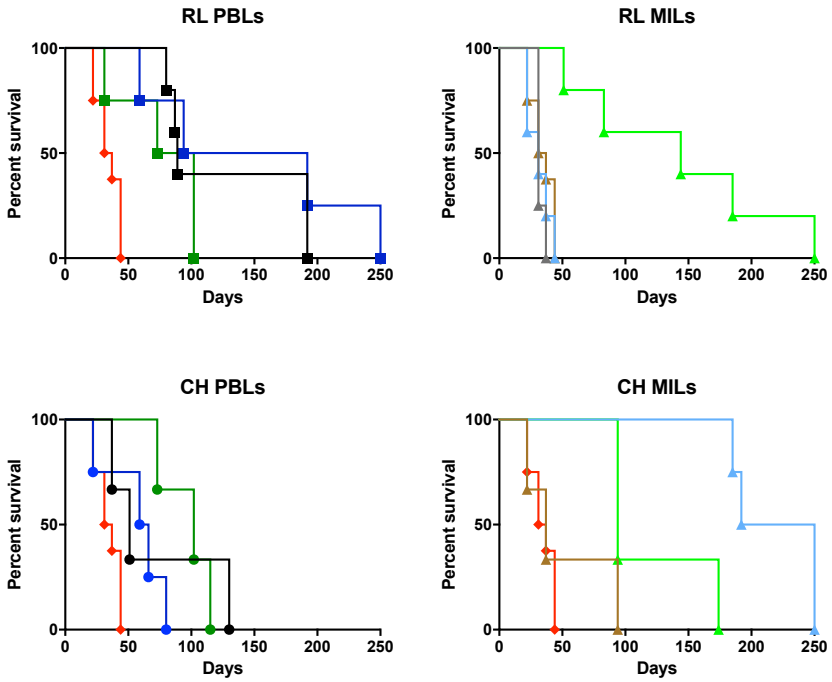
A)


No T-Cells
ng/mL IgE
Days Post T-Cells
RL PBL
RL BM
CH PBL
CH BM
WT
EV
BB

B)


No T-Cells
RL WT
PBL (4 mice)
BM (4 mice)
RL Empty Vector
PBL (4 mice)
BM (5 mice)
RL Switch Receptor
PBL (4 mice)
BM (5 mice)
CH WT
PBL (4 mice)
BM (5 mice)
CH Empty Vector
PBL (4 mice)
BM (4 mice)
CH Switch Receptor
PBL (4 mice)
BM (4 mice)
ng/mL IgE
Days Post T-Cells

C)


RL PBLs
RL MILs
CH PBLs
CH MILs
Percent survival
Days
0
50
100
150
200
250

D)

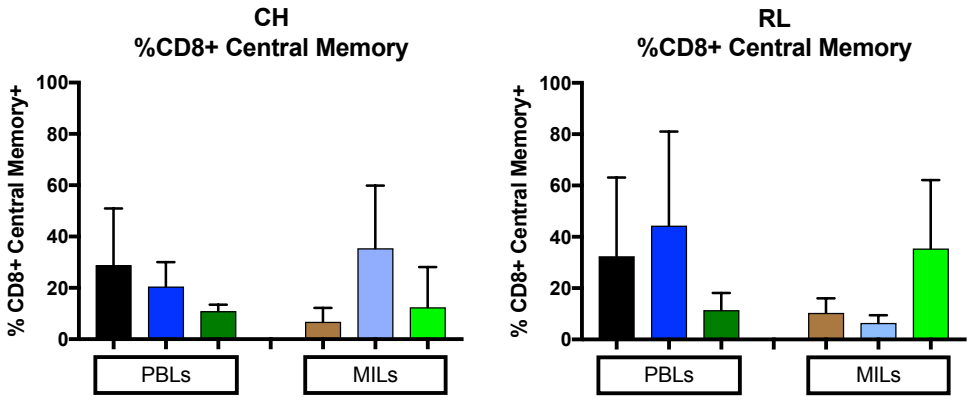


Figure 16: In-vivo tumor burden and survival may correlate with CD8+ central memory T-cells. IgE ELISAs using serum collected from mice were performed to determine the turmor burden in mice. **A)** Comparison of tumor burden in mice receiving no T-cells, MILs or PBLs (WT, EV or PD1BB switch receptor). **B)** Tumor burden in mice receiving no T-cells or WT, EV or PD1BB switch receptor MILs or PBLs. **C)** Survival of mice recieing no T-cells, MILs or PBLs (WT, EV or PD1BB switch receptor) **D)** Flow cytometry was used to determine the percentage of CD8+ central memory cells as determined by CD45RO+ CD62L+ CD8+ cells.

While in-vivo survival is certainly important to examine, looking at why mice died is also important. All of the mice that did not receive T-cells died of tumor burden (Figure 17). The majority of mice that received MILs also died of tumor, while surprisingly the majority of mice receiving PBLs died of GVHD. As was previously mentioned, this could be because PBLs are more polyclonal than MILs. Having a

broader T-cell repertoire may bring with it a higher risk for alloreactivity. While this is a potential concern when working with cell lines and mice, this may not be a problem clinically because a patient's own T-cells are used for transplant.

All PBLs demonstrated unexpected efficacy in contrast to WT MILs. However, there is some concern that this activity may be due to an alloreaction and not tumor specificity. However, the PD1BB switch receptor MILs exhibited improved anti-tumor cytotoxicity, suggesting that a PD1BB switch receptor does improve the ability of MILs to persist and kill tumor in-vivo as well as in-vitro. Additionally, survival seems to correlate with T-cell cytotoxicity as well as the number of CD8+ central memory cells in-vivo. This data suggests that a PD1BB switch receptor MIL is worth consideration to improve the efficacy of MILs in a clinical setting.

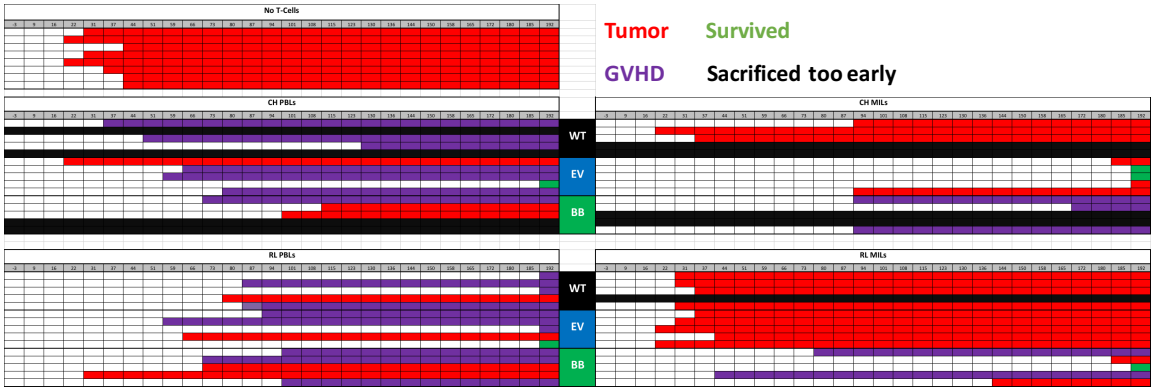


Figure 17: Causes of death during in-vivo experiment. Cause of death was determined for each mouse during the in-vivo experiment. Death due to tumor burden (red) was determined by the presentation of hind leg paralysis or visible tumor growth and high tumor burden by IgE ELISA. Death due to GVHD (purple) was determined by a lack of detectable tumor by IgE ELISA, weight loss, fur loss and lethargy. Excluded mice (black)

were due to sacrificing mice prematurely. Several mice were sacrificed at the end of the experiment but did not have tumor and were not sick (green).

REFERENCES

1. Mullard, A. FDA approves first CAR T therapy. *Nat. Rev. Drug Discov.* **16,** 669–669 (2017).

2. Lesokhin, A. M. *et al.* Nivolumab in patients with relapsed or refractory hematologic malignancy: Preliminary results of a phase ib study. *J. Clin. Oncol.* **34,** 2698–2704 (2016).

3. Mazo, I. B. *et al.* Bone marrow is a major reservoir and site of recruitment for central memory CD8 + T cells. *Immunity* **22,** 259–270 (2005).

4. Di Rosa, F. & Pabst, R. The bone marrow: A nest for migratory memory T cells. *Trends Immunol.* **26,** 360–366 (2005).

5. Noonan, K. *et al.* Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. *Cancer Res.* **65,** 2026–2034 (2005).

6. Noonan, K. A. *et al.* Adoptive transfer of activated marrow-infiltrating lymphocytes induces measurable antitumor immunity in the bone marrow in multiple myeloma. *Sci. Transl. Med.* **7,** 288ra78 (2015).

7. Pianko, M. J. *et al.* Efficacy and toxicity of therapy immediately after treatment with nivolumab in relapsed multiple myeloma. *Leuk. Lymphoma* **0,** 1–4 (2017).

8. Ocio, E. M. *et al.* Pembrolizumab (Pembro) plus lenalidomide (Len) and low-dose dexamethasone (Dex) for relapsed/refractory multiple myeloma (RRMM): Efficacy and biomarker analyses. *J. Clin. Oncol.* **35,** 8015 (2017).

9. Schlenker, R. *et al.* Chimeric PD-1:28 receptor upgrades low-avidity T cells and restores effector function of tumor-infiltrating lymphocytes for adoptive cell therapy. *Cancer Res.* **77,** 3577–3590 (2017).

10. Tang, X. *et al.* The advantages of PD1 activating chimeric receptor (PD1-ACR) engineered lymphocytes for PDL1(+) cancer therapy. *Am. J. Transl. Res.* **7,** 460–73 (2015).

11. Liu, X. *et al.* A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Res.* **76,** 1578–1590 (2016).

12. Prosser, M. E., Brown, C. E., Shami, A. F., Forman, S. J. & Jensen, M. C. Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. *Mol. Immunol.* **51,** 263–72 (2012).

13. Yi, K. H. *et al.* Adoptively transferred tumor-specific T cells stimulated ex vivo using herpes simplex virus amplicons encoding 4-1BBL persist in the host and show antitumor activity in vivo. *Cancer Res.* **67,** 10027–37 (2007).

14. Song, D. G. *et al.* In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). *Cancer Res.* **71,** 4617–4627 (2011).

15. Gerner, M. Y., Heltemes-Harris, L. M., Fife, B. T. & Mescher, M. F. Cutting edge: IL-12 and type I IFN differentially program CD8 T cells for programmed death 1 re-expression levels and tumor control. *J. Immunol.* **191,** 1011–5 (2013).

16. Shuford, W. W. *et al.* 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. *J. Exp. Med.* **186,** 47–55 (1997).

17. Zhang, N. & He, Y.-W. The antiapoptotic protein Bcl-xL is dispensable for the development of effector and memory T lymphocytes. *J. Immunol.* **174,** 6967–73 (2005).

18. Kroon, H. M. *et al.* 4-1BB Costimulation of Effector T Cells for Adoptive Immunotherapy of Cancer: Involvement of Bcl Gene Family Members. *J. Immunother.* **30,** 406–416 (2007).

19. Qin, J. Y. *et al.* Systematic comparison of constitutive promoters and the doxycycline-inducible promoter. *PLoS One* **5,** (2010).

20. Norrman, K. *et al.* Quantitative comparison of constitutive promoters in human ES cells. *PLoS One* **5,** (2010).

21. Bridgeman, J. S. *et al.* CD3zeta-based chimeric antigen receptors mediate T cell activation via cis- and trans-signalling mechanisms: Implications for optimization of receptor structure for adoptive cell therapy. *Clin. Exp. Immunol.* **175,** 258–267 (2014).

22. Abiko, K. *et al.* IFN-γ from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. *Br. J. Cancer* **112,** 1501–1509 (2015).

23. Garcia-Diaz, A. *et al.* Interferon Receptor Signaling Pathways Regulating PD-L1 and PD-L2 Expression. (2017). doi:10.1016/j.celrep.2017.04.031

24. Liao, Y. *et al.* Marked anti-tumor effects of CD8+CD62L+ T cells from melanoma-bearing mice. *Immunol. Invest.* **44,** 147–163 (2015).

25. Klebanoff, C. A. *et al.* Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. *Proc. Natl. Acad. Sci.* **102,** 9571–9576 (2005).

CIRRICULUM VITAE

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EDUCATION

Ph.D. **2017** **Cellular and Molecular Medicine**
Johns Hopkins School of Medicine Baltimore, MD
Dissertation: Flipping the Switch on PD-1 in Multiple Myeloma
Advisor: Ivan Borrello, M.D.

B.S. **2007** **Biology**
University of California, San Diego

RESEARCH EXPERIENCE

JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE
Ph.D. ***August 2012 to October 2017***
Baltimore, MD
Completed my graduate work in the lab of Ivan Borrello, M.D. in the department of Oncology and Tumor Immunology. My project involved determining role of PD-1 in multiple myeloma (MM) based on clinical data indicating that single agent PD-1 blockade yielded no benefit in patients with MM, potentially due to its expression on MM tumor cells. Another goal was to improve the ability of marrow infiltrating lymphocytes (MILs) to persist in-vivo while maintaining or increasing their intrinsic tumor-specificity. Specifically, I created a lentiviral PD-1 / 4-1BB switch receptor to effectively target blockade of naturally expressed PD-1 to T-cells, via competition with the switch receptor, while also providing an activating signal upon binding to its ligands, PD-L1 or PD-L2, on tumor cells.

ONYX PHARMACEUTICALS (Formerly Proteolix, Inc.)
Sr. Research Associate ***August 2007 to July 2012***
San Francisco, CA
Reported Shirin Kapur, Ph.D., Associate Director of Biology. I held a major role in developing a proteasome active-site subunit ELISA (ProCISE) used to measure pharmacodynamic (PD) activity of specific subunits of the proteasome. Processed PD clinical patient samples (blood, PBMCs and bone marrow) and most PD animal samples using biochemical assays as well as ProCISE for both carfilzomib (Kyprolis) and oprozomib (ONX-0912). Spearheaded a biomarker program for carfilzomib (Kyprolis) using plasma samples obtained from clinical trials looking at cytokines and growth factors as well as proteomics in collaboration with UCSF. Additionally, I researched the role of the immunoproteasome inhibitor, ONX-0914 (PR-957), in Treg differentiation and autoimmune diseases.

BIOGEN IDEC
Intern ***May 2006 to June 2007***
San Diego, CA
Reported to Veronica Gabarra-Neicko, Ph.D., Senior Scientist, Tumor Biology. Worked in a group focused on the importance of the integrin alpha-6/beta-4 in tumors.

UNIVERSITY OF CALIFORNIA, SAN DIEGO, SCHOOL OF MEDICINE
Lab Assistant ***April 2004 to Feb 2007***
San Diego, CA
Reported directly to Silvia Resta-Lenert, M.D., Ph.D., Assistant Professor in the lab of Kim Barrett, Ph.D.. Main project focused on the role of synbiotics (prebiotics + probiotics) and the upregulation of the MCT1 transporter protein (a transporter of butyrate). Performed western blots, immunoprecipitation, solution preparation and tissue culture. Co-authored papers and multiple abstracts (see 'Publications' below); presented abstract at April 2006 Experimental Biology Conference, San Francisco, CA

PUBLICATIONS

Lee, S.J., Borrello I.M., (2016) in Plasma Cell Dyscrasias (Cancer Treatment and Research). Aldo M. Roccaro, Irene M. Ghobrial Oct; 207-222

Lee SJ, Levitsky K, Parlati F, Bennett MK, Arastu-Kapur S, Kellerman L, Woo TF, Wong AF, Papadopoulos KP, Niesvizky R, Badros AZ, Vij R, Jagannath S, Siegel D, Wang M, Ahmann GJ, Kirk CJ. (2016) Clinical activity of carfilzomib correlates with inhibition of multiple proteasome subunits: application of a novel pharmacodynamic assay. Br J Haematol. Jun;173(6):884-95

Papadopoulos KP, Burris HA 3rd, Gordon M, Lee P, Sausville EA, Rosen PJ, Patnaik A, Cutler RE Jr, Wang Z, **Lee S,** Jones SF, Infante JR. A Phase I/II Study of Carfilzomib 2-10 min Infusion in Patients with Advanced Solid Tumors. (2013) Cancer Chemother Pharmacol. Oct;72(4):861-8

Badros AZ, Vij R, Martin T, Zonder JA, Kunkel L, Wang Z, **Lee S**, Wong AF, Niesvizky R. (2013) Carfilzomib in multiple myeloma patients with renal impairment: pharmacokinetics and safety. Leukemia. Aug;27(8):1707-14

Niesvizky R, Martin TG 3rd, Bensinger WI, Alsina M, Siegel DS, Kunkel LA, Wong AF, **Lee S**, Orlowski RZ, Wang M. (2013) Phase Ib dose-escalation study (PX-171-006) of carfilzomib, lenalidomide, and low-dose dexamethasone in relapsed or progressive multiple myeloma. Clin Cancer Res. Apr 15;19(8):2248-56

Ichikawa HT, Conley T, Muchamuel T, Jiang J, **Lee S**, Owen T, Barnard J, Nevarez S, Goldman BI, Kirk CJ, Looney RJ, Anolik JH. (2012) Beneficial effect of novel proteasome inhibitors in murine lupus via dual inhibition of type I interferon and autoantibody-secreting cells. Arthritis Rheum. Feb;64(2):493-503

Arastu-Kapur S, Anderl JL, Kraus M, Parlati F, Shenk KD, **Lee SJ**, Muchamuel T, Bennett MK, Driessen C, Ball AJ, Kirk CJ (2011) Non-proteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res. Mar 1

Parlati F, **Lee SJ**, Aujay M, Suzuki E, Levitsky K, Lorens JB, Micklem DR, Ruurs P, Sylvain C, Lu Y, Shenk KD, Bennett MK.. (2009) Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. Blood, Vol. 114, Issue 16, 3439-3447

PATENTS

Ivan M. Borrello, **Susan Lee**, Kimberly A. Noonan, Drew M. Pardoll. (2016) Marrow infiltrating lymphocytes (MILs) as a source of t-cells for chimeric antigen receptor (CAR) therapy

Ivan M. Borrello, **Susan Lee**, Kimberly A. Noonan, Drew M. Pardoll. (2016) Immune checkpoint chimeric antigen receptors therapy